The Spiracular Morphology of the Hawaiian Damselfly Megalagrion blackburni (Odonata)[1]

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INTRODUCTION

In the Odonata, growth and differentation is gradual throughout the nymphal stages although much of the final differentiation of adult structures takes place during the final (ultimate) instar. The environment shifts radically from water to air at the emergence of the adult and most differences in the respiratory system can be correlated with this change. Three stages can be observed: (1) the aquatic nymph, (2) the semi-aquatic nymph of the ultimate instar and (3) the terrestrial, air-breathing adult.

The major nymphal respiratory mechanism of the odonate nymph is the diffusion of oxygen through the surface of the body and the surface area may be increased by caudal lamellae (damselflies) or by a rectal branchial basket (dragonflies). The odonate adult has a generalized tracheal system open to the air through 10 pairs of spiracles. The semi-aquatic stage of the final instar retains the nymphal structures and has a single, functional pair of spiracles on the mesothorax. The major structural changes which take place between the nymph and the adult at final metamorphosis are (1) the loss of the caudal lamellae or rectal gills, (2) the shift from the single pair of functional spiracles of the ultimate instar nymph to the 10 pairs of functional spiracles of the adult and (3) the replacement of most of the cylindrical diffusion tracheae of the nymph by compressible, thin-walled air sacs in the adult.

The species of the endemic Hawaiian damselfly genus *Megalagrion* (family Coenagrionidae) are unique in that the nymphs represent a complete range of habitats from aquatic to terrestrial. The nymph of *M. oahuense*, and possibly an unidentified megapodagrionid nymph from New Caledonia (Willey, 1955) are the only Zygoptera which are terrestrial in the early instars. Williams (1936) has made a thorough study of the habitat

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and life history of each species of *Megalagrion*, and the taxonomy of the genus has been reviewed by Zimmerman (1948). The work presented here was undertaken as part of a study of the development of terrestrialism in the nymphs of this genus (Willey, 1956). In order to understand the significance of the structural modification of the nymphs from various habitats, it was necessary to make a detailed study of the generalized zygopteran respiratory system.

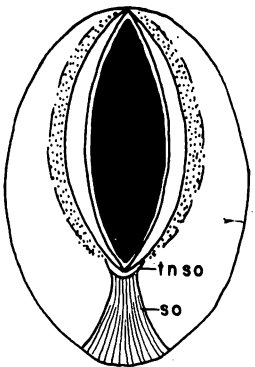
Materials and methods: The species chosen for the following study was *Megalagrion blackburni*. Its habitat represents what may be tentatively considered an intermediate in the wide range of environments represented by the nymphs of this genus and the greatest range and abundance of known instars were available for study. Occasionally, lack of proper material made it necessary to use specimens of *Megalagrion oceanicum*, an extremely closely related species.

Comparative studies were made of other odonate families. The results of the studies of *Basiaeschna janata* (Aeschnidae) will be reported only where necessary to demonstrate the structural modifications between the suborders Zygoptera and Anisoptera.

Structures were studied by gross dissection of fixed and fresh material and by serial sections. Specimens were fixed in Bouin's or Helley's fluids, embedded by Peterfi's celloidin-impregnation method (Pantin, 1948), and stained with Mallory's triple connective tissue stain, Mann's methyl blue-eosin stain, and Holmes' silver method (Holmes, 1947).

The odonate spiracle: The spiracles represent the sites at which the integument invaginated to form the tracheal system. The most primitive spiracles are, therefore, simple openings with no provisions for closing found in some of the Apterygota. However, most of the pterygote spiracles are equipped with mechanisms which control the size of the aperture. The external openings generally become secondarily invaginated to form a small pit or atrium. The original tracheal orifice (*t*) becomes the opening at the bottom of the atrium, and the atrial orifice (*a*) is the secondary external aperture. As a result, two types of spiracular closing mechanisms have developed; one located at the atrial orifice and the other at the tracheal orifice. In most insects the mechanism which controls the size of the external or atrial orifice by means of movable lips or flaps is found in the thorax and the internal type of closing apparatus is typical of the abdomen (Snodgrass, 1935). All 10 pairs of spiracles of the Odonata are modified lip or external types of closing apparatus (Figs. 1 to 6).

The presence of spiracles in the nymph has been known for a long time and the question of their function and structure has long been a subject of interest. The presence of 2 thoracic pairs and 7 pairs of abdominal spiracles was recognized in the late 18th century by Reaumur (1742), who studied their function by covering them with oil. The first abdominal spiracles are hidden so thoroughly by the fusion of the abdominal tergum



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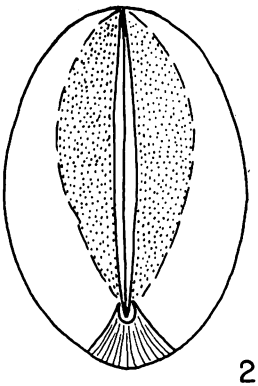

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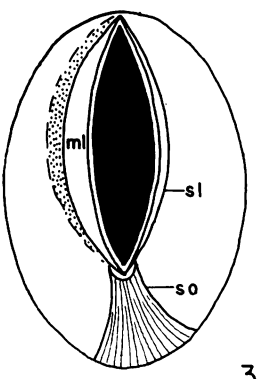

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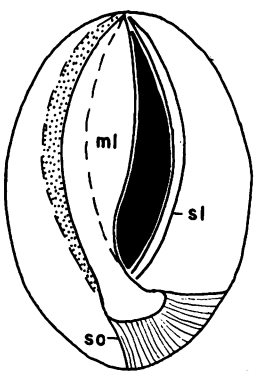

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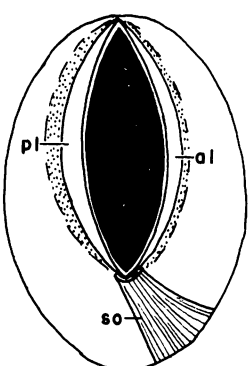

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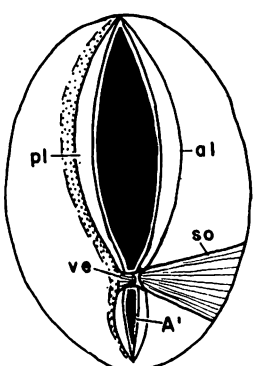

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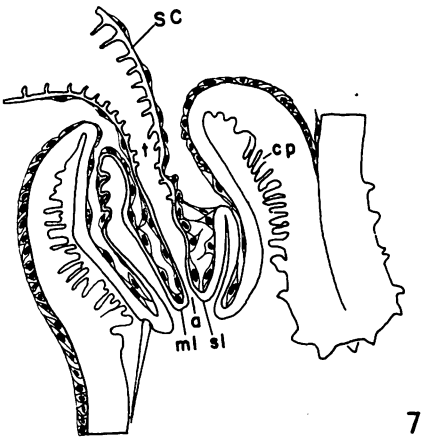
Diagrammatic representation of the typical spiracular structure.

FIG. 1. *Primitive spiracle in open position.*
FIG. 2. *Primitive spiracle in closed position.*
FIG. 3. *Abdominal spiracle of odonate adult.*
FIG. 4. *Metathoracic spiracle of odonate adult.*
FIG. 5. *Mesothoracic spiracle of odonate nymph.*
FIG. 6. *Mesothoracic spiracle of odonate adult.*

with the sternum, and the overlapping by the metathorax, that it was not recorded until later by Carus (1827) in his study of the blood circulation of the nymph of *Agrion puella.* Much controversy has revolved around the problem of the functional nature of structurally open but mechanically impervious spiracles in the odonate nymph (Hagen, 1880; Calvert, 1893; Miall, 1895; Koch, 1936; Naumann, 1951; Corbet, 1962). It is now generally accepted that the nymphal spiracles all remain nonfunctional except the mesothoracic spiracle (mesospiracle) which is functionally open during the ultimate instar. The mesospiracle can act to bring air into the tracheal system during periods of drought and during the transition period after the nymph leaves the water and metamorphoses into the aerial adult. Structural studies have been made of nymphal mesospiracles by Gericke (1917), and Snodgrass (1954), and the general structure of the thoracic as well as the abdominal spiracles was reviewed by Wolf (1935). The basic structure of the adult spiracle has been studied by Burmeister (1836), Landois (1867), Tillyard (1917), Maloeuf (1935) and Miller (1962). Identification of the thoracic muscles follows Clark's (1940) nomenclature, and of the abdominal muscles follows Snodgrass (1935) and Desai (1937).

The adult spiracles of the abdomen and metathorax: The abdominal spiracles exhibit the closest approach to the simple lip-closing spiracular mechanism (Fig. 3). The edges of the spiracular plate or peritreme (*dl*) become modified into two cuticularized lips or valves which originally were equal in size and shape. Each extends along 1/2 of the rim and meets the other across the orifice. The elasticity of the atrial walls and the lips serves to keep the spiracle open. Closure is accomplished by contraction of a small muscle, the spiracular occlusor (*so*), which inserts on a small plate or lobe in the atrial wall below the point where the lips are united. Contrary to Poonawalla's observations (1966), the spiracular occlusor was found in all abdominal spiracles in all species examined. Connective tissue fibers which run from the walls of the atrium to the lips in opposition to the spiracular occlusor, effect an efficient transfer of the tension from the muscle attachment on the atrial walls to the lips.

The following description is based on the 8th abdominal spiracle because it is the largest of the abdominal spiracles (Figs. 9, 10). The others are basically the same structure. This spiracle is encased in a protecting ovate cuticular plate which lies in the pleural membrane with its long axis parallel to that of the abdomen. As a result of the overlap of the enlarged adult tergites over the narrow sternites, the abdominal spiracle actually lies with its atrial orifice in a dorsal position (Fig. 8). Therefore, the spiracular connective leading from the lateral trunk must run ventrad and around dorsad to attach to the under (ventral) side of the spiracle. Such a sharp curve in the trachea, in addition to the pulsating movements of the abdomen, certainly must have a ventilating effect at the spiracle. The anterior tergo-pleural muscles (*dvm*) serve to maintain the



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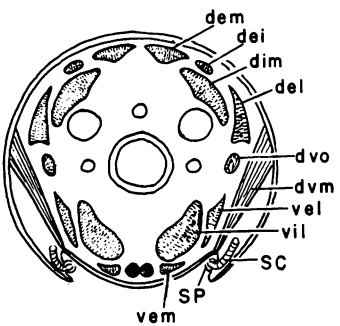

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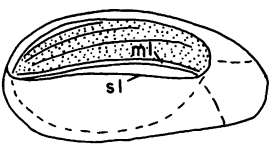

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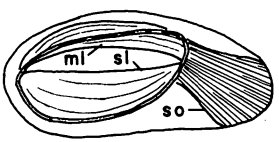

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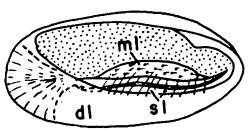

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0.5 mm. (Fig. 11)

0.25 mm. (Figs. 9,10)

0.1 mm. (Fig. 7)

Abdominal spiracle.

FIG. 7. Megalagrion blackburni. *Section of adult spiracle of the 6th abdominal segment.*

FIG. 8. *Diagram of cross section of adult abdomen.*

FIG. 9. M. blackburni. *External view. Adult 8th abdominal spiracle.*

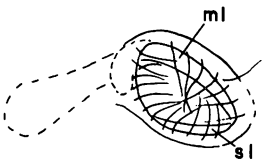
FIG. 10. M. blackburni. *Internal view. Adult 8th abdominal spiracle.*

FIG. 11. Basiaeschna janata. *External view. Adult 8th abdominal spiracle.*

infolded position of the sternum. The close proximity of the edges of the tergites and sternites, coupled with the constant ventilating movements, have caused interesting modifications in the cuticle of the tergum and sternum, which probably serve to overcome friction and to prevent adhesion of the 2 surfaces (Fig. 7). The exocuticle has increased in depth and has developed many perpendicular processes or interconnected ridges which do not allow any 2 flat, uniform surfaces to come together which might adhere to each other.

The abdominal spiracle (Fig. 3) is not quite as symmetrical as the generalized spiracle proposed by Snodgrass (1935) (Fig. 1). The muscle which originates on the rim of the peritreme has changed the angle of its pull slightly and, as a result, has transferred much of the tension and action to one lip. The muscle shift is the most common modification of this type of spiracle and may become quite extreme in more complex structures, such as the mesospiracle of the adult. Even though the shift is very slight in the abdominal spiracle, the lips have differentiated enough to distinguish the relatively stationary lip (*sl*) from the movable one (*ml*). Contraction of the spiracular occlusor will pull against the elasticity of the movable lip and cause it to straighten out and meet the stationary lip. Opening is simply achieved through the inherent elasticity of the lip and its attachments to the other lip. Setae, sensory in function (Miller, 1962), are found on the lips and the rim of the spiracular plate in *Basiaeschna*, but only on the rim in *Megalagrion*.

In the adult metathoracic spiracle (Figs. 12 and 13), the muscle has shifted laterally, and the movable lip has expanded into a flap. In this structure can be seen an advance in the trend of changes which began in the abdominal spiracle. However, the basic structure is very much the same. Just a change in the direction of pull of the spiracular occlusor, which pulls one lip against the other, insures a more complete closure of the atrial orifice. The closing action of the movable lip is no longer solely a matter of the deformation of the flexible lip. It is the rotation of a flap in which the inherent elasticity of its attachment at either end serves to restore it to the open position when the muscle relaxes. The cuticular plate on which the spiracular occlusor is inserted is enlarged and fused with the end of the movable lip to form a knob. Miller (1962) has shown a similar arrangement in *Aeschna juncea* and *Ictinogomphus perox*. It may have been this structure which led Krancher (1881) to describe an internal closing mechanism in which a lever squeezes the tracheal orifice shut. However, the pull of the metaspiracular occlusor rotates the flap so that it falls across the opening and lies with its edge against the immovable lip which has fused secondarily in *Basiaeschna* with the pleural cuticle (Fig. 14). The movable lip in *Basiaeschna* is covered abundantly with fine setae which may serve as a filter as well as sensory receptors. In *Megalagrion*, the filter setae are located on the cuticular rim of the pleuron around the spiracle (Fig. 12).



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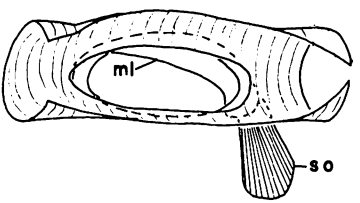

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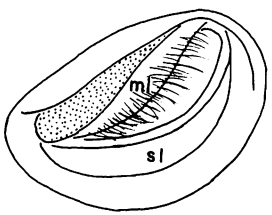

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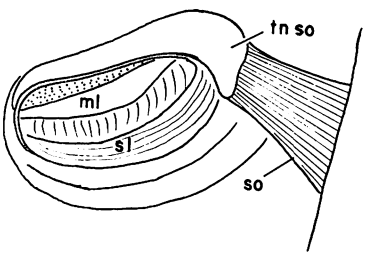

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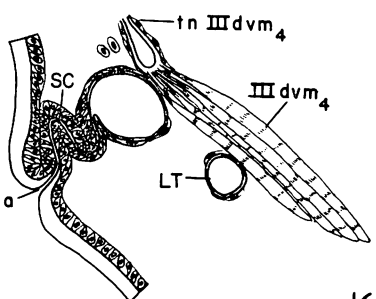

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0.5 mm. (Figs. 14,15)

0.25 mm. (Figs.12,13)
0.2 mm. (Fig.16)

Metathoracic muscle.

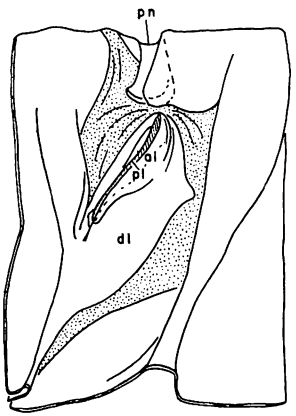
FIG. 12. Megalagrion blackburni. *External view.*
FIG. 13. M. blackburni. *Internal view.*
FIG. 14. Basiaeschna janata. *External view.*
FIG. 15. B. janata. *Internal view.*
FIG. 16. M. blackburni. *Section through nonfunctional spiracle of nymph.*

The Nymphal Spiracles of the Metathorax and Abdomen: The nonfunctional aspect of these organs is evident from their structure which is very similar to each other. The intima of the metathoracic spiracular connective is very thin and is collapsed. The connective is folded in the small space between the integument and the lateral trunk which is held close to the pleuron by the body of the metathoracic accessory depressor (III dvm_4). The part of the spiracular connective which lies against the integument is fused to the hypodermis at its basement membrane (Fig. 16). The lips have not differentiated from the peritreme cuticle. The spiracles open through the integument by a very narrow slit through which the tracheal intima is withdrawn at each molt. The spiracular connective of the abdominal spiracle is also collapsed. In the posterior segments (5th through 8th), the connective runs anteriorly from the spiracle to the lateral tracheal trunk and is folded against the body wall by the delicate tergo-sternal muscle (*dvm*) (Fig. 28). In the anterior abdominal segments, however, the spiracles are tucked in the pleural-intersegmental folds. The tergosternal muscles maintain the pleural fold similar to the adult position. The collapsed condition of the connective, the envelopment by the abdominal folds, and the sharp bends in the connective as it passes around the folds all aid to keep the small patent spiracular slit impervious under normal conditions. The cuticle in this area is extremely hydrophobic. In the experiments of Dewitz and others, in which the solubility coefficient of the gas is reduced in the water by heating or adding alcohol, a bubble develops in the supersaturated medium at the spiracle and rapidly increases in volume until it rises to the surface (Koch, 1934). Therefore the appearance of bubbles under such conditions is only an indication of the open, rather than functional, qualities of the spiracle.

The spiracular muscles in the abdomen and the metathorax are not present in functional form in the nymph. They are a loose bundle of homogeneous fibers which are smooth, lack obvious transverse striations and are similar to those described by Cremer (1934). The fibers are probably non-contractile, but they do maintain a slight tension because they are connected to the cuticle by very long, attenuated hypodermal cells.

The nymphal mesospiracle: The functional mesospiracles of the ultimate instar nymph are quite prominent in the dorso-lateral part of the intersegmental membrane just beneath the end of the prothoracic postnotum. When the nymph breathes air, the pronotum moves forward exposing the membrane and the spiracular lips which rise up and become prominently erect. The lips do not open wide, as in the adult, but only enough to allow the passage of air past the internal cuticular meshwork which lines the atrium. Viewed from above, the opening in the spiracles is seen as a silver line which is the exposed part of the atrial lining.

The size of the opening of the nymphal mesospiracle diminishes progressively in size in successively earlier instars. In *Megalagrion blackburni*,



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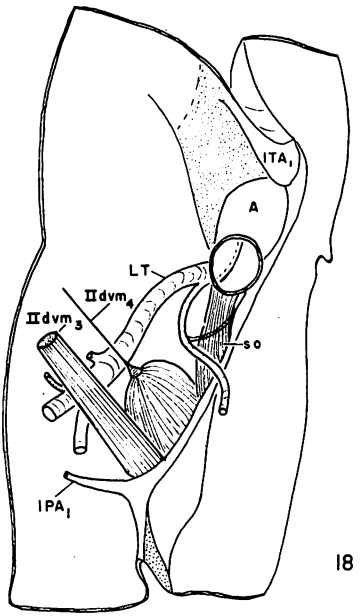

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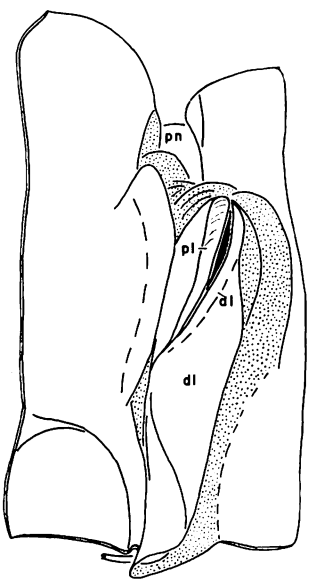
0.5 mm. (Figs. 17, 18)
1 mm. (Figs. 19,20)



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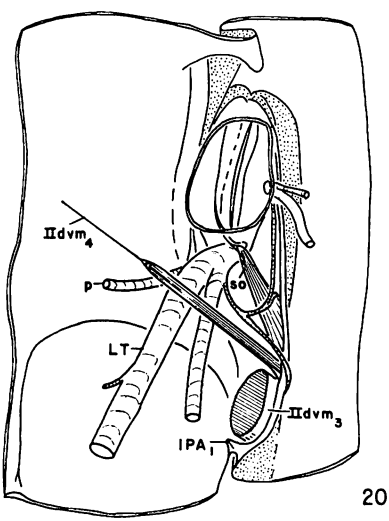

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Mesothoracic spiracle of final instar nymph.

FIG. 17. Megalagrion blackburni. *External view.*
FIG. 18. M. blackburni. *Internal view.*
FIG. 19. Basiaeschna janata. *External view.*
FIG. 20. B. janata. *Internal view.*

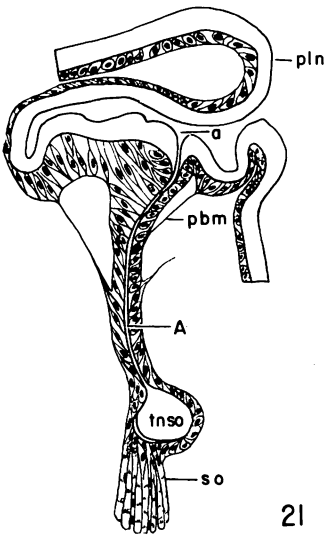
the mesospiracular opening is 0.4 mm. long in the ultimate instar and ranges down through 0.15 mm, 0.12 mm, 0.075 mm, 0.05 mm, and 0.05 mm, successively, from the penultimate to the 6th instar before the ultimate stage. It is interesting to note that a similar reduction occurs in the ultimate instars of the species of *Megalagrion* ranging from the largest in the aquatic species, *M. xanthomelas*, to the smallest in the terrestrial species, *M. koelense*.

The basic structure of the nymphal mesospiracle is similar to the abdominal spiracle (Figs. 3 and 5). Both lips are flexible and movable, but the slight angle of the pull of the spiracular occlusor causes more movement in the posterior lip. The spiracular occlusor originates on the infolded preepisternum or spiracular plate, and inserts ventrally on the atrium at the junction of the lateral trunk from the mesothorax (Fig. 18). The anterior lip is thin and concave; at its median end it curves over the end of the dorsal lip and is continuous with the intersegmental membrane. The posterior lip is like a slightly twisted, flattened club which is attached laterally on the edge of the pleuron and medially within the fold of the anterior lip (Fig. 17).

The membrane along the edge of the posterior lip allows free movement except at the ventral tip where the lip is firmly attached to the anterior edge of the pleuron. Whenever the spiracle is opened, the pronotum moves forward, partly due to the pull of the coxal promotors ($Idvm_{2,3}$), and exposes the whole structure. When the spiracular occlusor is relaxed, the lips erect as a result of their elastic attachments and the tension created in the intersegmental membrane which tightens as the pronotum moves forward. The spiracular plate is also pulled forward and bent at the point of junction of the posterior lip with the pleuron.

Closure of the spiracle is accomplished by two processes: (1) relaxation of elements already under tension and (2) active tension created by other muscles. The pronotum closes over the intersegmental membrane in response to the pull of the prothoracic dorsal longitudinal muscles; the tensions existing in the spiracular plate are relaxed, and the spiracle tends to rotate back toward the pleuron. Coupled with this action is the pull of the spiracular occlusor which adds to the rotation and closure of the lips. Active contraction of the prothoracic intersegmental muscle (*Iism*) dorsal to the spiracle and the mesothoracic anterior depressor ($IIdvm_3$) pull the intersegmental membrane and the spiracular plate down and further depress the lips. They complete the folding under of the spiracular plate as the pronotum closes over it.

In *Basiaeschna* nymphs, the mesospiracle is attached to the pleuron along most of its length and the intervening membrane is greatly reduced (Figs. 19 and 20). The action of the spiracular plate, which occurs in *Megalagrion*, is reduced in *Basiaeschna* to the infolding of the ventral part of the plate due to the pull of the accessory depressor muscle ($IIdvm_4$). The



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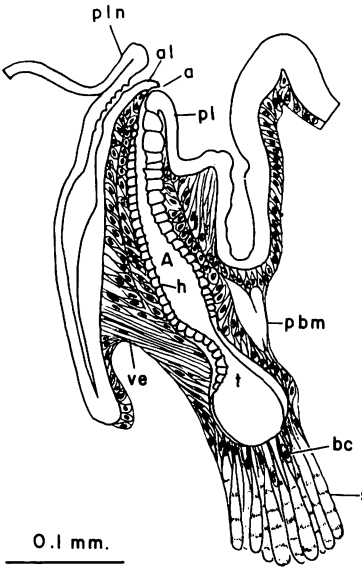

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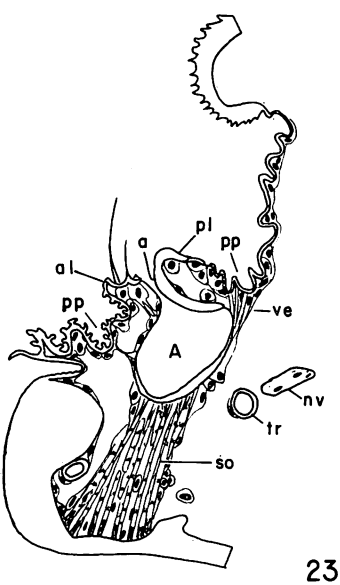
0.1 mm.

Figs.21,22,23



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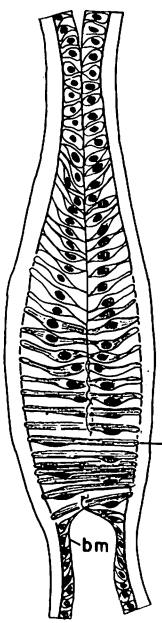

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Mesothoracic spiracle of *Megalagrion blackburni.*

FIG. 21. *Penultimate nymph (nonfunctional).*

FIG. 22. *Final instar nymph (functional).*

FIG. 23. *Adult*

FIG. 24. *Diagrammatic section through the wing pad to show position of connective tissue fibers and hypodermal cells*

FIG. 25. Megalagrion blackburni. *Nonfunctional mesothoracic spiracle of penultimate nymph.* ×190.

trend results in increased fusion and stability of the spiracular plate with a corresponding increase in the importance of the inherent elasticity of the posterior lip to open the spiracle. The anterior lip is united into the spiracular plate which reduces its action to the minimum. The contractions of the prothoracic dorsal muscles (*Idvm*) and the intersegmental muscle (*Iism*)are still exceedingly important to the closing mechanism of the spiracle.

Since the odonate spiracles have the external lip type of closing mechanism, the atrium is a secondary invagination, and its lining is more closely related to the integument rather than the tracheal intima. The nymphal mesospiracle has a very characteristic atrial intima (Figs. 22 and 29), a honeycomb-shaped lining which is not derived from the taenidia, since it lines just the atrium (*sc*) and stops at the tracheal orifice. This occurs both in *Megalagrion* and *Basiaeschna*. The intima is a specialization of the exocuticle which develops during the pre-molt growth of the penultimate nymph. As the posterior lip rotates back during the opening of the spiracle, the first cells of the lining are exposed and fill the atrial orifice. They are probably the structures which Landois (1867) described as a sound-producing comb. This characteristic nymphal structure is not carried over into the adult. It probably serves to prevent sudden water penetration as well as prevent excessive dehydration of the spiracular cells.

The odonate spiracle is closed by the action of two lips, but the attachment of the occlusor muscle in both nymph and adult is on a small cuticularized plate in the bottom of the atrium. The tension from the muscle contraction is transferred to the lips by connective tissue fibers. If there were no mechanism for the transfer of muscle tension to the spiracular lips, the effect of the muscle would only deform the atrium and might even enlarge the atrial orifice by drawing in the integument at the edge of the opening. However, connective tissue fibers connect the walls of the atrium with the pivot point (*pp*) of the lip and thereby create a discrete lip structure, with the integument as the outer surface and the atrial wall as the inner surface (Figs. 22 and 23). A few strands of this type are found in the nonfunctional nymphal mesospiracles, but they are most highly developed in the functional spiracle of the ultimate instar nymph and the adult.

These connective tissue fibers are formed from hypodermal cells under a tension which may be created by muscle contraction or growth and structural development. Similar fibers have been found in developing wings (Marshall, 1915; Schlüter, 1933; Richards, 1951), the caudal gills of damselflies, and even in the carapace of the crustacean *Panulirus argus* (Travis, 1955). Beckel (1955) observed that the opening muscle of *Hyalophora cecropia*, which had been described by many previous authors, is actually a very long bundle of connective tissue fibers which oppose the pull of the spiracular occlusor. The differentiation of the hypodermal cells seems to depend a great deal on the tension present. In areas where the tension

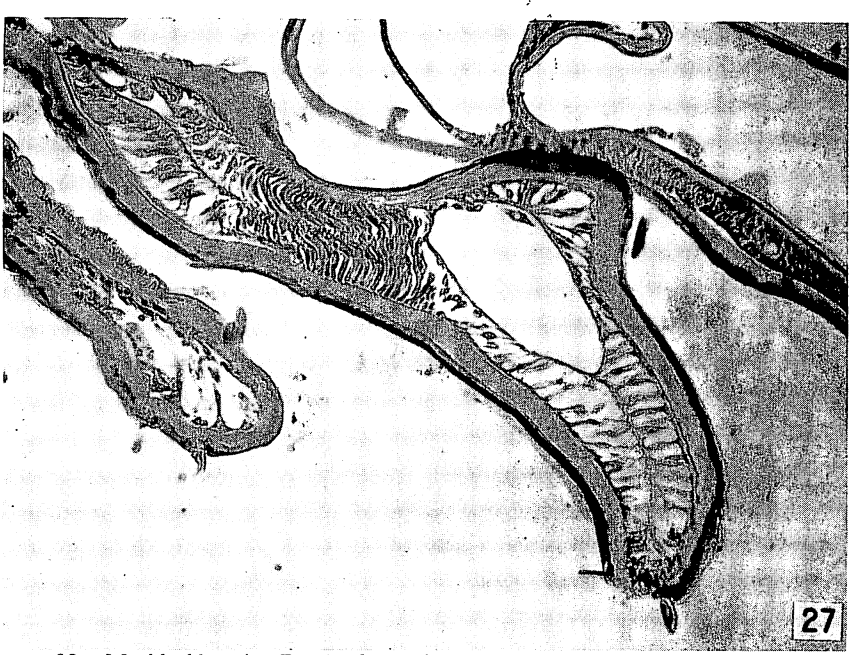
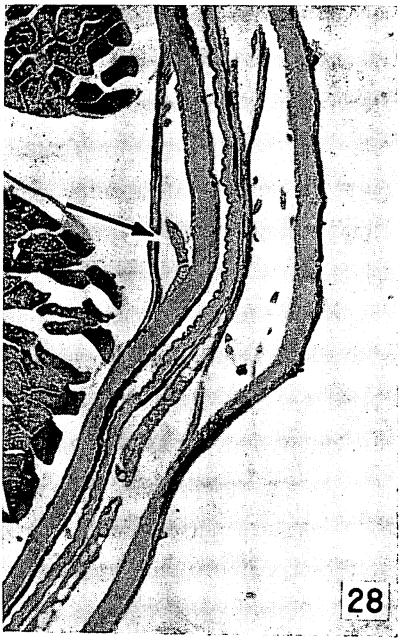


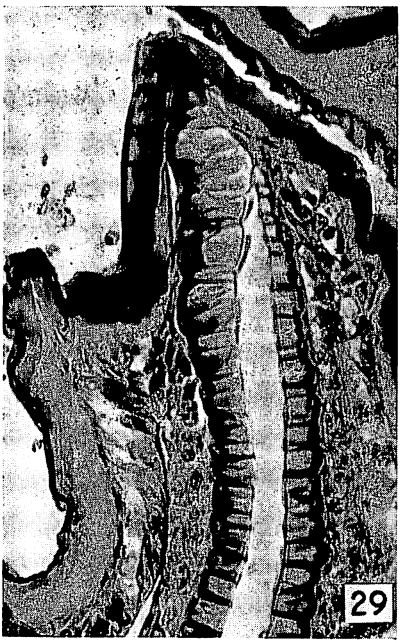
FIG. 26. M. blackburni. *Functional mesothoracic spiracle of final instar nymph.* ×180.
FIG. 27. M. oceanicum. *Cross section of wing pad to show position of connective tissue fibers and hypodermal cells.* ×160

is uneven, as in the spiracular lips, the whole series of cell types are present (Figs. 24 and 27); whereas in areas in which the tension is uniformly great, such as in the apodemes in the adult metanotum, the fibers represent the same level of development.

When two hypodermal cell layers come together, as in an invagination or fold, they lie parallel to each other with their basement membranes pressed together. As they are drawn apart again, the hypodermal cells become very attenuated, whereas the basement membranes tend to remain attached to each other. The nuclei migrate toward the center of the cell and then distally until they lie near the basement membrane. At the same time, the thin cellular strands which have remained attached to the cuticle thicken, develop intracellular fibrillae and develop a strong foot-like attachment to the cuticle. Opposing cells across the basement membrane become firmly attached together, so that when the membrane breaks, they remain attached. In areas of greater tension, the basement membrane tends to disappear, and the connected cells fuse into a single unit in which the fibrils extend their full length from cuticle to cuticle. In highly developed stages, the connective tissue fibers are homogeneous, thick, and fibrous, with only occasional indications of a basement membrane. Each fully developed fiber seems to have only a single ovate nucleus. However, it is possible that there are two, one at each end. Marshall (1915), in his work on the wing of *Platyphylax*, found that some of the fiber nuclei migrate into the region of the basement membrane just before the fusion of the two hypodermal cells and disintegrate there.

At each molt, a larger spiracular structure is developed which is accompanied by tensions produced by expansion during molt. In the penultimate instar of *Megalagrion*, long strands of basement membrane, sometimes accompanied by one or more long, attenuated cells, run from the collapsed walls of the spiracular connective (atrium) to the hypodermis of the integument (Figs. 21 and 25). The pattern of their attachment is fairly consistent. The closer to the tracheal orifice these strands are attached, the further away on the integument they are attached. Similarly, when arising from the atrial hypodermis near the atrial orifice, they run to an attachment on the integument closer to the edge of the spiracular lip. In addition, as the atrial hypodermis approaches the integumental hypodermis near the edge of the lip, the basement membrane is fused, and attenuated hypodermal cells stretch across the narrow space. Evidently, during the premolt period of the previous instar, as the hypodermal cells multiplied, the layers of the atrium and the integument were crowded close together. At molt, the expansion of the cuticle and the spreading of the cells created tensions which pulled the two epithelial layers apart. Such a process is indicated by the progressively longer strands which occur farther down the atrium. In the nonfunctional penultimate spiracle no definitive connective tissue fibers have developed.





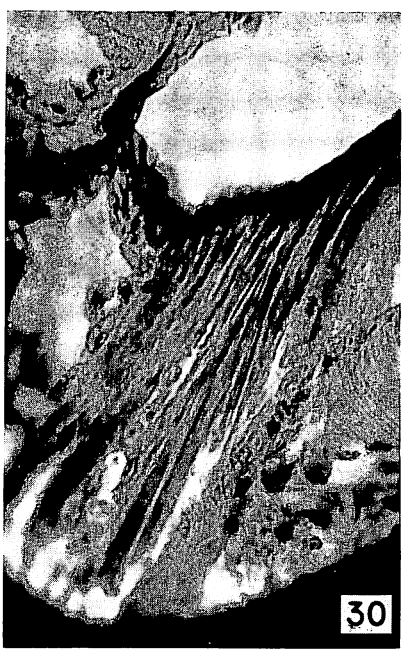


FIG. 28. Megalagrion blackburni. *Section through spiracular connective (arrow) of 7th abdominal segment of final instar nymph.* ×130.

FIG. 29. M. blackburni. *Honeycomb lining of atrium of mesospiracle of final instar nymph.* ×570.

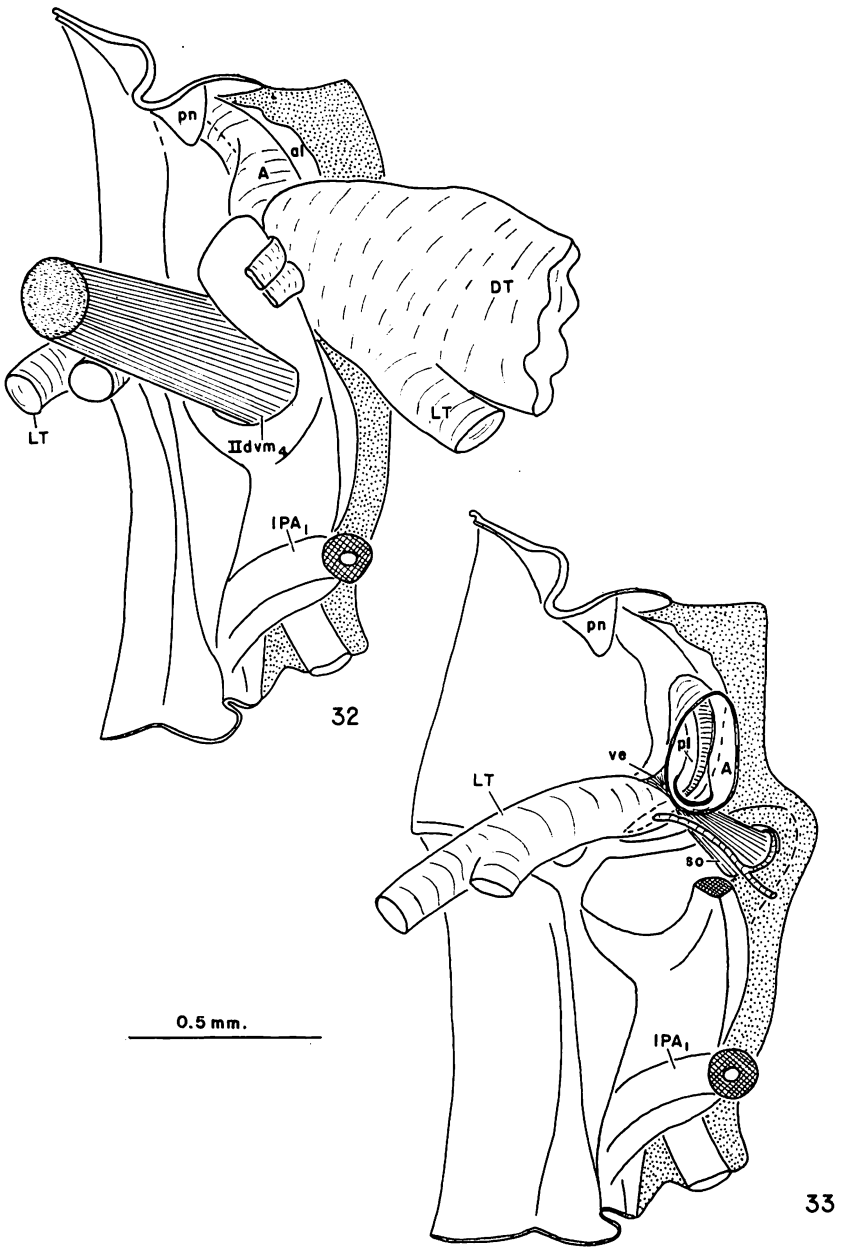
FIG. 30. M. blackburni. *Spiracular occlusor of adult mesothoracic spiracle.* ×570.

FIG. 31. Basiaeschna janata. *Functional mesothoracic spiracle of final instar nymph.* ×410.

Since the mesospiracle is functional in the ultimate instar nymph, the lips have elongated to produce an elevated closing mechanism (Figs. 22 and 26). With the growth of the lips, the two hypodermal layers have come together almost as far as the tracheal orifice. As a result, tension produced by the spiracular occlusor has resulted in the successive development of the fibers. The greatest development is in the anterior lip, which is not so flexible as the posterior lip, and therefore produces greater resistance to the muscle contraction. As a result, the atrial and integumental hypodermis are united to produce a lip which acts as a single unit, and the pivot points are generally coincidental with the attachment sites of the longest connective tissue fibers.

The adult mesospiracle: The mesospiracle of the adult is the most highly modified from the generalized spiracular structure (Figs. 6, 23 and 38). The opening is larger, and the spiracular muscles are attached almost at right angles to the lips (Fig. 33). The original atrium is very reduced, and the two pockets lying below the atrial opening originate from the tracheae. The spiracular opening is enlarged by the ventral extension of the atrial orifice to open over a portion of the lateral trunk, which is held against the integument by the accessory depressor muscle (*IIdvm*$_4$). The remnants of the nymphal atrium are found only in the dorsal atrium of the adult from which the tracheal trunks run anteriorly to the head and posteriorly into the mesothorax as the dorsal trunk. The honeycomb lining of the nymphal atrial wall is not present in the adult. The atrial intima in the *Megalagrion* adult is not modified in any way. In *Basiaeschna*, many dendritic processes are found lining the wall which are extensions of the taenidia. These act as filters in the absence of specialized setae and spines which are found on the anterior lip of the mesospiracle in *Megalagrion*. A filter apparatus is almost always present; but its form, whether as an internal modification of the atrial wall or as external setae and spines, seems to have been developed independently. The nymphal mesospiracle exhibits an internal honeycomb lining of the atrium, whereas the adult *Megalagrion* mesospiracle has lip setae in contrast to the taenidial processes of *Basiaeschna*. In the metaspiracle, the setae are located on the pleuron in *Megalagrion*, whereas they cover the movable lip in *Basiaeschna*. In the abdominal spiracles, the lip setae form the filter apparatus as well as sensory receptors of both odonate groups.

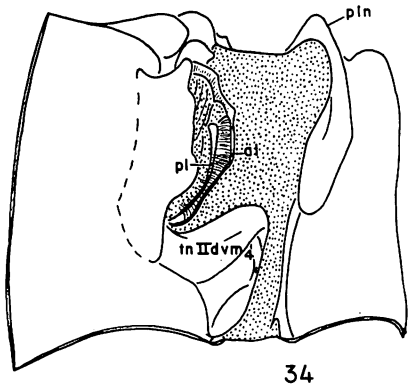
The adult muscles of all the spiracles are very similar. They are formed of short, loosely connected muscle fibers which do not run absolutely parallel (Fig. 30). Other cells may be found between the fibers, many of which are blood cells. Beckel (1955) described the same sort of muscle in the abdominal spiracle of *Hyalophora cecropia*. He also found many clusters of cells along the muscle fibers associated with nerve endings. The muscle fibers are made up of solid bundles of fibrils similar to the nymphal locomotory muscles. The mesospiracular muscle of the ultimate instar



Adult mesothoracic spiracle of *Megalagrion blackburni.*

FIG. 32. *Internal lateral view.*

FIG. 33. *Internal lateral view with shield removed.*



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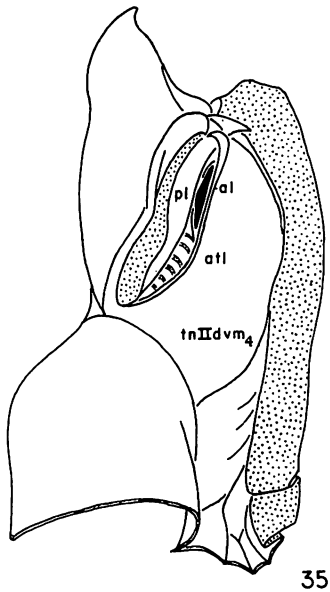

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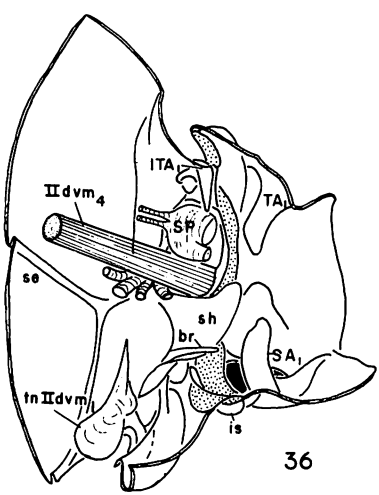
1mm. (Figs. 34,35,37)
2mm. (Fig. 36)



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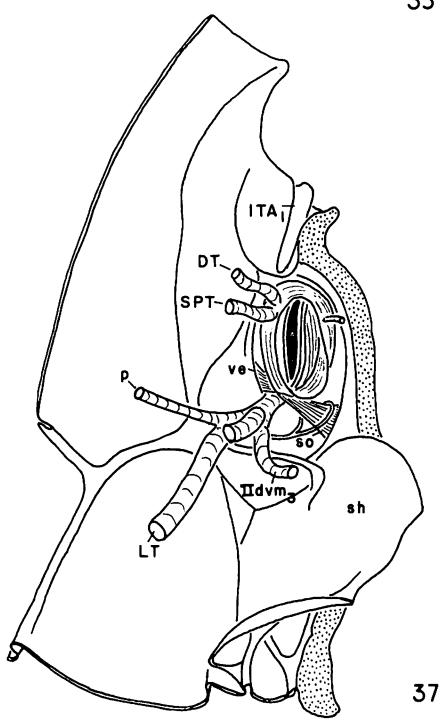

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Adult mesothoracic spiracle.

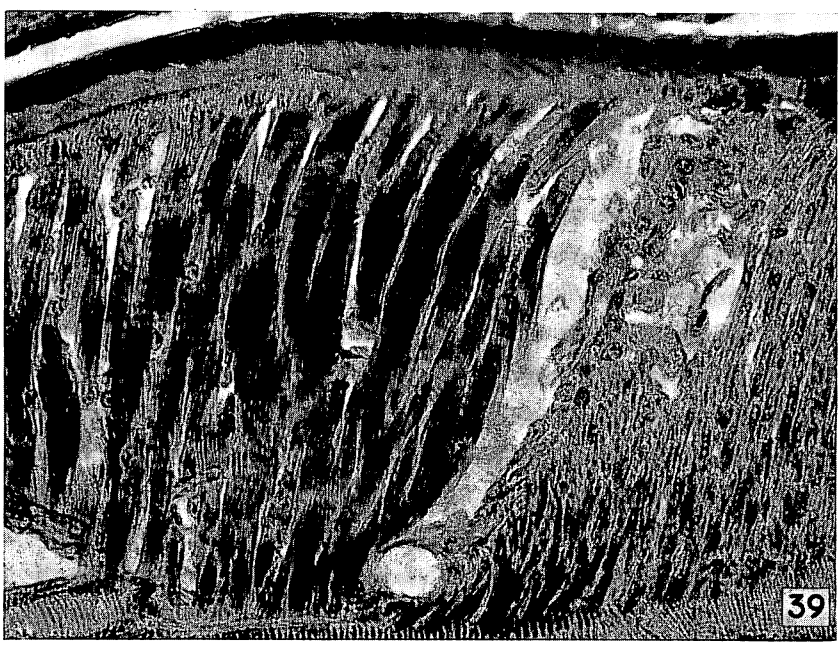
FIG. 34. Megalagrion blackburni. *External view.*
FIG. 35. Basiaeschna janata. *External view.*
FIG. 36. B. janata. *Internal view.*
FIG. 37. B. janata. *Internal view.*

nymph is the same as in the adult. The muscles of the abdominal and metathoracic spiracles are also the same in the last part of the ultimate instar during which they complete their differentiation. It is very possible that the mesospiracular muscle in very early instars is nonfunctional. However, even in such an early stage as the 6th instar before the ultimate instar, this muscle appears transversely striated and similar in every way, except size, to the ultimate instar.

The spiracular occlusor seems to stretch across the atrium to attach on the movable (posterior) lip. However, as in the nymph, it is attached to the bottom of the atrium at the junction with the lateral trunk. The ventral extension of the atrial orifice creates the false appearance that the muscle has shifted its point of insertion. The increase in the angle of contraction of the spiracular occlusor has transferred the closing movements entirely to the posterior lip. The stationary (anterior) lip in *Megalagrion* is very stiff and acts as a bow against which the posterior lip closes (Fig. 34). In *Basiaeschna*, the anterior lip is fused directly with the spiracular plate (Fig. 35). The movable lip opens by its inherent elasticity and its attachment with both ends of the anterior lip.

The increase in angle of the spiracular occlusor is due to the dorsal movement of part of the spiracular plate (*dl*) which accompanied the growth of the adult anterior depressor muscle ($IIdvm_3$). As the depressor muscle increased in diameter, it partially enclosed the point of origin of the accessory depressor ($IIdvm_4$) and caused the dorsal movement of this small muscle. The spiracular plate, on which the accessory depressor originates, moved up to an antero-ventral position in *Megalagrion* with respect to the spiracle (Fig. 33), whereas that of *Basideschna* spread up anteriorly to the spiracle and fused with the anterior lip, forming the characteristic peritreme plate of the Anisoptera (Figs. 35). In spite of the varying extent to which the spiracular plate has changed, the spiracular occlusor has shifted dorsally to the same degree in both *Megalagrion* and *Basiaeschna*. The difference in extent in the spiracular plates in the two groups may be due to the difference in copulatory positions. In *Megalagrion*, the semi-forked superior caudal appendages of the male clasp the posterior lobe of pronotum of the female. The distal part of the male superior appendage is held under the pronotum by the spiracular plate of the female which presses against it. The characteristic anterior position of the heavy spiracular plate in the damselfly helps maintain contact with the male and, at the same time, leaves the spiracle free to function. In *Basiaeschna*, the superior appendages of the male clasp the female behind the head and there is no contact with the spiracular mechanism at all.

The accessory depressor ($IIdvm_4$) has its origin just lateral to that of the spiracular occlusor and runs postero-dorsad across the ventral atrium of the spiracle. Thus it can regulate air flow into the lateral trunk of the mesothorax. Similarly, the anterior depressor muscle ($IIdvm_3$) runs to the



Megalagrion blackburni

FIG. 38. *Mesothoracic spiracle of adult.* ×105.

FIG. 39. *Comparison of adult (left) and nymphal (right) muscle in the nymph.* ×540.

pteronotum over the accessory muscle, and the contraction of the two muscles must be simultaneous. In *Megalagrion*, the shield of the intersegmental pleural apophysis is greatly expanded to accommodate the large base of the anterior depressor muscle (*IIdvm*$_3$) (Fig. 32). It is flexible and exhibits rhythmic movement as the flight muscle contracts. The top of the shield is curved and lies against the large anterior tracheal trunk just before it divides into the dorsal and lateral trunks of the prothorax. Thus, these collapsible tracheal vessels lie between the pulsating base of the accessory depressor and the dorsal longitudinal muscles. Fraenkel (1932) reports that Odonata show little or no change in ventilatory movements at the onset or during flight. At the beginning of flight, the mesothoracic, metathoracic, and first abdominal spiracles open instantly and remain open throughout flight (Miller, 1962). The ventilative action of the depressor muscles on the tracheal trunk directly at the base of the atrium may be completely adequate during flight to supply oxygen to the thorax and the head.

The expansion of the shield does not occur in *Basiaeschna* (Fig. 36). Probably, this difference in the size of the shield is primarily related to the copulatory position or the more extreme skewness in the damselfly thorax and is only secondarily related to the respiratory mechanism. In the same way, the spiracular plate is markedly different in the two groups, but the spiracular occlusors have the same relative positions. Any conclusions based purely on anatomy must necessarily be hypothetical. The function of the expanded shield in relation to the respiratory mechanisms must be examined physiologically for clarification.

CONCLUSION

The respiratory system of the zygopterous odonate exhibits changes during the transition from the aquatic nymph to the aerial adult. The early instar nymph has essentially a closed tracheal system, but the ultimate instar develops a functional pair of primitive mesospiracles and may obtain oxygen directly from the air. These spiracles allow the nymph to resist drought, migrate a considerable distance from the water for the imaginal emergence and to feed on prey close to the water's edge. All 10 pairs of spiracles are present in the nymph but they function solely as the site at which tracheal intima is withdrawn at each molt.

The abdominal and metathoracic spiracles of the adult as well as the nymphal mesothoracic spiracle have the simple external lip type of closing apparatus. The contraction of the occlusor muscle tends to deform or rotate the lips against each other and thus close the orifice. When the occlusor relaxes, the lips spring open due to inherent elasticity. Such an indirect closing action is not as efficient as that found in the adult mesothoracic spiracle in which contraction of the occlusor pulls one lip directly across the orifice to press against the other lip. The change in angle of

pull is due primarily to the shift in position of the perispiracular sclerites in response to the skewing of the adult thorax which accomodates the development of flight muscles and wing attachments.

The pull of the spiracular occlusor is transferred to the spiracular lip by connective tissue fibers. These fibers are common in the odonate thorax and are formed from hypodermal cells which are under tension; these cells then fuse and develop intracellular fibrillae. Commonly described in the wingpads and caudal lamellae, the fibers are also found among the apodemes and in the spiracular lips.

Respiration in the insect is not controlled by the spiracles and tracheae alone. Muscular action is very important in spiracular control and ventilation. The comparatively flexible thorax and thoracic muscles of the nymph are efficiently utilized to provide secondary controls on the closure of the functional mesothoracic spiracles and prevent water penetration into the open tracheal system. The mesothoracic accessory depressor ($IIdvm_4$) which originates on the preepisternal plate draws in the spiracular plate of the functional nymphal mesospiracle to fit under the pronotum. In the metathorax, the accessory depressor ($IIIdvm_4$) presses the spiracular connective of the nonfunctional metaspiracle against the integument to prevent strain on the atrial orifice which might result in water penetration. Similarly the ventral and dorsal longitudinal muscles and the intersegmented muscles of the pro- and mesothorax hold the nymphal sternites together and draw the pronotum down over the spiracular apparatus. In the adult, however, movement of these muscles ventilate the tracheae within the rigid, box-like thorax rather than seal them away from the external environment. Similarly, the tergosternal muscles aid in the closure of the nymphal abdominal spiracles and in ventilation of the adult spiracles.

The interrelationships between the many structures involved in the spiracular respiratory mechanism are complex. The shift of environment from the nymph to the adult takes place throughout the final instar of the nymph. By varying changes of position, the spiracular mechanism has maintained its basic structural pattern and, at the same time, has accomplished the necessary changes in function accompanying the change of environment.

It is with just such a complex of respiratory structures that nymphs of the Hawaiian genus *Megalagrion* have penetrated into a series of habitats ranging from aquatic to terrestrial. It is not difficult to suggest that the final instar of the odonate nymph shows a sort of preadaptation to a terrestrial mode of life due to its functional mesothoracic spiracles. However the presence of earlier instar damselfly nymphs in terrestrial habitats is exceptionally rare. The genus *Megalagrion* is ideally suited to study the problems of terrestrial adaptation by the "strong comparative inference" method (Klopfer and Hailman, 1967, p. 189) which derives data of phylogenetic significance from a natural adaptive series. A thorough knowledge

of the detailed structure of the whole adaptive complex will lead to pertinent experimentation which can provide an understanding of nymphal evolution in the Odonata.

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KEY TO ABBREVIATIONS

Numerals refer to the body segment.

a	atrial orifice
A	atrium
al	anterior lip
atl	atrial lining
bc	blood cell
bm	basement membrane
br	bridge of IPA
cp	cuticular process
dei	dorsal exterior intermediate muscle
del	dorsal exterior lateral muscle
dem	dorsal exterior median muscle
dim	dorsal interior median muscle
dl	spiracular plate
DT	dorsal tracheal trunk
dvm	dorso-ventral muscle
dvo	dorso-ventral oblique muscle
h	honeycomb of atrium
IPA	intersegmental sterno-pleural apodeme
is	intersternite
ITA	intersegmental tergal apodeme
LT	lateral tracheal trunk
ml	movable lip
n	nerve
p	pedal trachea
pbm	process of basement membrane
pl	posterior lip
pln	posterior lobe of postnotum
pn	postnotum
pp	pivot line of spiracular lip
SA	sternal apophysis
SC	spiracular connective
se	sterno-episternal apodeme
sh	shield of IPA
sl	stationary lip
so	spiracular occlusion
SP	spiracle

SPT spiracular trunk
t trachea
tn attachment of muscle
ve connective tissue fibers
vel ventral exterior lateral muscle
vil ventral interior lateral muscle